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Occurrence and biodegradability studies of selected pharmaceuticals and personal care products in sewage effluent☆

Jim T. Yu [a], *Edward J. Bouwer* [a,*], *Mehmet Coelhan* [b]

[a] *Department of Geography and Environmental Engineering, Johns Hopkins University, 313 Ames Hall/3400 North Charles Street, Baltimore, MD 21218, United States*

[b] *Technical University of Munich, 85350 Freising-Weihenstephan, Germany*

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ABSTRACT

It is important to determine the behavior of constituents in treated sewage during soil aquifer treatment for water recycling. A collaborative project with Herman Bouwer published in 1984 documented the fate and occurrence of Priority Pollutants during groundwater recharge using wastewater with application to water reuse and agricultural systems. New compounds are continually being manufactured and released to the environment. The interest today has shifted to the behavior of emerging contaminants. One important class of emerging contaminants is pharmaceuticals and personal care products (PPCPs). The hazards to the environment and human health of trace levels of PPCPs in water supplies are poorly understood. A multi compound method using solid phase extraction and chemical derivatization with pentafluorobenzylbromide, followed by analysis via gas chromatography/mass spectrometry was used to study the occurrence and removals of 18 PPCPs in a local wastewater treatment plant (WWTP). Overall, 16 out of the 18 selected PPCPs, which span a range of therapeutic classes and some commonly used personal care products, were detected in raw sewage samples collected from the Baltimore Back River WWTP. Ten of the 18 selected PPCPs were detected in the treated sewage effluent, signifying incomplete removal for the majority of the PPCPs during the wastewater treatment processes. The majority of the target analytes were detected in both the influent and effluent WWTP samples at μg/L levels, although some PPCPs (e.g., naproxen and ibuprofen) were encountered at μg/L levels. Biodegradation is an important process for wastewater and soil aquifer treatment. Aerobic batch biodegradation (using activated sludge as microbial inocula) experiments were conducted to gain insight into the biodegradation behavior of our target PPCPs at initial concentrations of 50, 10, and 1 μg/L. Sterile control studies showed no loss of our target PPCPs during the entire incubation period, and sorption to the biomass was found to be negligible for all testing conditions. Biodegradation results were not sensitive to the initial concentration and showed that 13 of the 18 PPCPs tested exhibited biotransformations greater than 80% after 50 days of incubation. Phenytoin, 5-fluorouracil, and diclofenac were the only three compounds with removals less than 60%. The occurrence study revealed the presence of PPCPs in sewage effluents, and the biodegradation study suggests that biotransformation is a possible removal mechanism for PPCPs during groundwater recharge or soil aquifer treatment for water recycling.

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* *Corresponding author*. Tel.: +1 410 516 7437; fax: +1 410 516 8996.
E-mail address: bouwer@jhu.edu (E.J. Bouwer).

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1. Introduction

Water is an essential component for ecosystem function and human existence. As the U.S. population continues to increase, greater demands are being placed on securing adequate water supplies, and greater challenges arise in purifying water of poorer quality for reuse (Bouwer, 1994, 2000, 2002). As we look forward, shortages of available water will increase as demand for water increases. One approach to augment available water supplies is to employ water recycling and reuse (National Research Council, 1998). Water recycling and reuse is being accomplished through aquifer storage recovery (ASR), groundwater recharge with treated wastewater, use of nonpotable water for irrigation of agricultural lands, golf courses and parks, and injection of treated water for preventing salt water intrusion. ASR refers to the purposeful recharge and storage of water in an aquifer system during times when water is plentiful, and recovery of the stored water during times when it is needed (Pyne, 1995). It is important to determine the behavior of constituents in source waters during water recycling and reuse to insure the production of high quality water and to reduce impediments to public acceptance of these alternative water management strategies. The principles of subsurface flow and environmental aspects of groundwater are addressed in a classic book authored by Bouwer (1978).

Much of the research in the 1970s and 1980s on behavior of trace compounds in water supplies focused on 129 chemicals listed as Priority Pollutants (Watts, 1998). The U.S. Environmental Protection Agency developed this list of chemicals based on their occurrence in water, chemical stability and structure, amount of chemical produced, hazardous nature, and availability of chemical standards and measurement. A collaborative project with Herman Bouwer published in 1984 documented the fate and occurrence of Priority Pollutants during groundwater recharge using wastewater with application to water reuse and agricultural systems (Bouwer et al., 1984). Samples of the wastewater applied to spreading basins and of renovated water taken from monitoring wells were characterized for priority pollutants using gas chromatography/mass spectrometry. Nonhalogenated aliphatics and aromatic hydrocarbons exhibited concentration decreases of 50–99% during soil percolation. Halogenated organic compounds were generally removed to a lesser extent. Chlorination of the wastewater had no significant effect on concentrations and types of trace organic compounds.

Volatilization was an important removal mechanism for the low-molecular weight compounds in the spreading basins; between 30 and 70% of the chlorinated benzenes and 1- and 2-carbon halogenated organic compounds were removed in this way. Biodegradation and sorption processes were likely responsible for the decreased organic concentrations resulting from soil passage. Several of the priority pollutants were detected in the groundwater during rapid infiltration, indicating such systems should be designed to localize contamination of the aquifer.

New compounds are continually being manufactured and released to the environment. Environmental contamination by pharmaceuticals and personal care products (PPCPs) has recently gained widespread public attention as a pervasive problem (Erickson, 2002a, 2002b). To date, more than 70 different compounds have been detected in surface and groundwater in the U.S., often at concentrations in the 0.01–1 μg/L range (Daughton and Ternes, 1999; Halling-Sorensen et al., 1998; Heberer, 2002; Jones et al., 2001; Kolpin et al., 2002). Municipal wastewater can be an important resource, but its use must be carefully managed to prevent adverse health effects and contamination of receiving waters (Bouwer, 2002; National Research Council, 1998; Stackelberg et al., 2004). Issues related to PPCP contamination will only gain in prominence as wastewater recycling becomes more prevalent; in some states the fraction of wastewater that is presently reclaimed is as high as 80% (United States Geological Survey, 2003). Many PPCPs possess acute toxicities similar to industrial chemicals, exhibit chronic toxicity even at concentrations below 1 μg/L (Bisceglia and Roberts, 2006), and exhibit ecotoxic effects principally through endocrine disruption (Sharpe and Irvine, 2004; Xia et al., 2005). Concern is also mounting that low-level contamination by certain PPCPs may contribute to the spread of antibacterial resistance (Hileman, 2005; Lear et al., 2002).

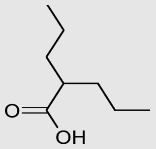
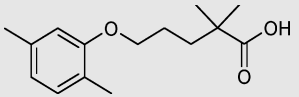
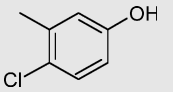
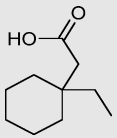
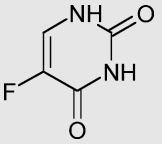
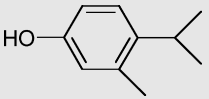
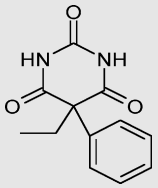
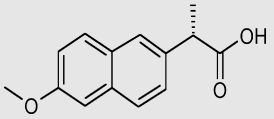
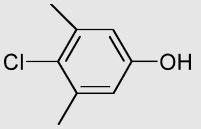
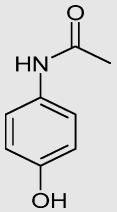
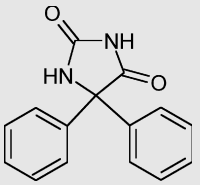
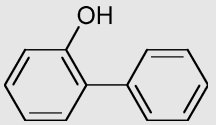
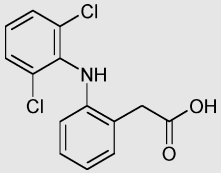
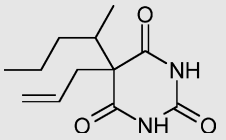
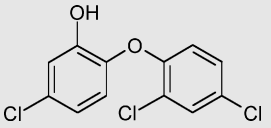
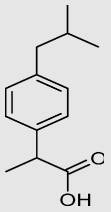
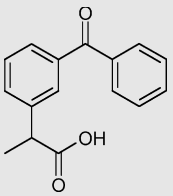
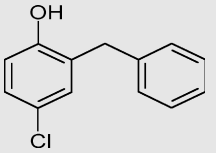
Whether PPCPs pose a substantial public health threat is a question that has not been fully resolved. The issue of PPCPs as environmental contaminants is likely to grow in prominence as future drinking water shortages force municipalities to begin reclaiming water. Yet, limited occurrence and removal data exist for PPCPs in municipal wastewater treatment plants, and the factors influencing their removals are seldom studied. For this special publication, we present our occurrence and removal study involving 18 PPCPs (Table 1, most of which have not been studied by other researchers) in the Back River wastewater treatment plant (BRWWTP) in Baltimore, MD. Biodegradation is an important removal process during wastewater treatment and for the purification of water through soil passage and groundwater recharge (e.g., river-bank filtration and soil aquifer treatment). Aerobic batch biodegradation experiments were conducted to gain insight into the biodegradation behavior of our target PPCPs at initial concentrations of 50, 10, and 1 μg/L.

2. Experimental

2.1. Chemicals and reagents

Reference standards for valproic acid [99-66-1; 2,2-di-*n*-propylacetic acid] and phenytoin [57-41-0; 5,5-diphenyl-2,4-imidazolidinedione] were purchased from Aldrich-Fluka (St. Louis, MO). Biosol [3228-02-2; 3-methyl-4-isopropylphenol], biphenylol [90-43-7], *p*-chloro-*m*-cresol [59-50-7], *para*-chloro-*m*-xylenol [88-04-0], ibuprofen [15687-27-1; 2-(4-isobutylphenyl)propionic acid], gabapentin [60142-96-3; 1-(aminomethyl)cyclohexaneacetic acid], acetaminophen [103-90-2; *N*-(4-hydroxyphenyl) acetamide], gemfibrozil [25812-30-0; 2,2-dimethyl-5-(2,5-xylyloxy)valeric acid], chlorophene [120-32-1; *o*-benzyl-*p*-chlorophenol], naproxen [22204-53-1; 2-naphthaleneacetic acid], triclosan [3380-34-5; 5-chloro-2-(2,4-dichlorophenoxy)phenol], ketoprofen [22071-15-4; 2-(*meta*-benzoylphenyl) propionic acid], and diclofenac [15307-86-5; 2-(2,6-dichlorophenyl)aminophenyl acetic acid] were purchased from Spectrum Chemical

Table 1 – Structures, chemical abstracts service registry number (CAS), and therapeutic classes for the target PPCPs selected for this study

Compound (CAS), therapeutic class	Structure	Compound (CAS), therapeutic class	Structure	Compound (CAS), therapeutic class	Structure
Valproic acid (99-66-1) *Anticonvulsant*		Gemfibrozil (25812-30-0) *Antilipemic Agent*		*p*-Chlorom-cresol (59-50-7) *Antiseptic Agent*	
Gabapentin (60142-96-3) *Anticonvulsant*		5-Fluorouracil (51-28-8) *Antineoplastic Agent*		Biosol (99-66-1) *Antiseptic Agent*	
Phenobarbital (50-06-6) *Barbiturates*		Naproxen (22204-53-1) *NSAID*		*p*-Chloro-*m*-xylenol (88-04-0) *Antiseptic Agent*	
Acetaminophen (103-90-2) *NSAID*		Phenytoin (57-41-0) *Anticonvulsant*		Biphenylol (90-43-7) *Antiseptic Agent*	
Diclofenac (15307-86-5) *NSAID*		Secobarbital (76-73-3) *Barbiturates*		Triclosan (3380-34-5) *Antiseptic Agent*	
Ibuprofen (15687-27-1) *NSAID*		Ketoprofen (22071-15-4) *NSAID*		Chlorophene (120-32-1) *Antiseptic Agent*	

(Gardena, CA). Secobarbital [76-73-3; 5-allyl-5-(1-methylbutyl) barbituric acid] and phenobarbital [50-06-6; 5-ethyl-5-phenylbarbituric acid] were obtained from Cambridge Isotope Laboratories (Andover, MA). 5-Fluorouracil [51-21-8; 5-fluoropyrimidine-2,4-dione] was obtained from ICN Biomedical Inc. (Irvine, CA).

Chemicals used for derivatization and extraction were HPLC-grade acetonitrile, HPLC-grade methanol, American Chemical Society reagent grade sulfuric acid, sodium thiosulfate, cyclohexane, pentafluorobenzyl bromide (PFBBr), and potassium carbonate. These chemicals were obtained from Sigma–Aldrich-Fluka (St. Louis, MO). Milli-Q water, prepared from deionized distilled water passed through a Milli-Q Plus UV (Millipore, Billerica, MA) water purification device, was used in all cases where high purity water was required. Trace nutrients used for batch biodegradation experiments were KH_2PO_4, K_2HPO_4, Na_2HPO_4, $FeCl_3$, NH_4Cl, $MgSO_4$ and $CaCl_2$, all obtained from Sigma–Aldrich–Fluka (St. Louis, MO).

2.2. Water sample collection and storage

Twenty-four hours influent and effluent composite samples were collected from the BRWWTP in Baltimore, MD. The BRWWTP receives about 180 million gal/day of wastewater from about 1.3 million residents. The plant is designed to achieve biological nutrient removal (BNR facility) with a solids residence time between 8 and 10 days for the bioreactors. Bleach is used for disinfection, and sodium bisulfite is added for dechlorination prior to effluent discharge. All of the solids produced are thickened and anaerobically digested. Influent and effluent wastewater samples were collected in 2.5 L amber glass bottles and transferred from the BRWWTP to our laboratory in coolers packed with ice and salt. Samples were stored at 4 °C overnight and extracted the next morning using the procedure described in the next section.

2.3. Solid phase extraction (SPE) procedure

Wastewater samples were filtered using 1.2 μm Millipore GF/C filters (Bedford, MA) after reaching room temperature. The water sample was then acidified to pH 2 by adding 2 M H_2SO_4. Prior to extraction, Phenomenex Strata X (500 mg) SPE cartridges were first conditioned with 5 mL of methanol, allowing the sorbent to soak in methanol without any vacuum applied. After contact with methanol for about 3 min, 5 mL of pH 2 Milli-Q water was passed through the cartridge by gravity for additional conditioning of the cartridge. Then, wastewater samples (250 mL for influents or 500 mL for effluents) were passed through the SPE cartridges at a flow rate of approximately 6 mL/min using a 12-fold vacuum manifold. After the extraction, the cartridges were washed with 5 mL of 10% methanol in Milli-Q water before elution of our target analytes with 7 mL of acetonitrile by gravity into a 20 mL Fisher Scientific borosilicate glass test tube with threaded end (Fair Lawn, NJ).

2.4. Derivatization procedure

After elution of the SPE cartridge with 7 mL of acetonitrile into a 20 mL test tube, 6 mL of Milli-Q water were then added to create the optimum reaction solvent for chemical derivatization. The derivatization procedure required addition of 180 μL of 10% K_2CO_3 (adjusted to pH 10.5) to the water acetonitrile solvent mix. Then, 70 μL of PFBBr was added, and the entire test tube was vortexed for 30 s. The test tube was capped with a Fisher rubber-lined cap (Fair Lawn, NJ), and the solution heated for 1 h at 100 °C in an oven. After cooling the solution to room temperature, 200 μL of 2 M sodium thiosulfate was added to react with excess PFBBr. The test tube was reheated for 10 min at 60 °C to insure the destruction of excess PFBBr. Then, 300 μL of cyclohexane (containing internal standards, 4,4-di-*tert*-butylbiphenyl-d_5 and 1-phenylnonane-d_3, at 100 μg/L) was added and the mixture vortexed for 1 min to extract the derivatized compounds. Finally, the test tube was nearly completely filled with Milli-Q water containing 5% NaCl for better phase separation. The top cyclohexane layer was transferred into a 100 μL Kimble glass insert (Vineland, NJ) for GC/MS analysis.

2.5. Determination of sample concentration

A five-point standard calibration curve for each PPCP was generated by spiking the Milli-Q water with the target analytes and two internal standards, 4,4-di-*tert*-butylbiphenyl-d_5 and 1-phenylnonane-d_3. Internal standards were used to normalize the peak area from one sample to another. Concentrations in the samples were calculated by first computing the peak area ratios of the target analytes and internal standards. Subsequently, each peak area ratio was then compared to the corresponding peak area ratio of known target analyte concentration and internal standards in the standard calibration curve to calculate the actual concentration. For determining the concentrations of PPCPs in wastewater samples, an additional solvent blank was used as a control and two spiked (~500 ng/L) recovery samples were extracted and derivatized identically to the other samples for the determination of percent recoveries. The analyte concentrations were then adjusted with corresponding recovery efficiencies obtained in the same matrix to provide accurate quantification of the target PPCP concentrations.

2.6. Instrumentation

GC/MS measurements were performed using a ThermoQuest (San Jose, CA) Trace 2000 gas chromatograph with a quadrupole mass spectrometer. Two microliters of each sample were injected using a programmed temperature vaporization injector (PTV) maintained at 250 °C, and the injection was under splitless mode for one minute and split mode afterwards. A DB-5MS (J&W; Palo Alto, CA) 30 m length × 0.25 mm ID × 0.25 μm phase thickness column was used for compound separation. The GC temperature program was: 105 °C for 1 min, 8 °C/min to 285 °C and then hold for 10 min. Mass spectra were obtained in electron impact mode (70 eV) with selected ion monitoring. Data were processed using *Xcalibur*™ software (Thermo Electron Corporation, San Jose, CA).

2.7. Biodegradation experiments

Short-term batch biodegradation tests were conducted by dividing the target PPCPs into two groups: drugs and antiseptics. The microbial inoculum for the biodegradation studies consisted of 1 mL of 1:1000 diluted waste activated sludge obtained

from the BRWWTP. All glassware and media were autoclaved, and solutions of the PPCPs were filter sterilized. Sterilization of different materials and chemicals were performed separately. In addition, the PPCP batch study was carried out in different individual bottles. About 100 mL of growth media were placed in 250 mL Erlenmeyer flasks, and the microbial inoculum was added in test samples, but not in controls. The growth media consisted of Milli-Q water containing trace nutrients (8.5 mg/L KH_2PO_4, 21.75 mg/L K_2HPO_4, 33.4 mg/L Na_2HPO_4, 0.25 mg/L $FeCl_3$, 2.7 mg/L NH_4Cl, 22.5 mg/L $MgSO_4$ and 27.5 mg/L $CaCl_2$). Sterile controls were also poisoned with mercuric chloride (~10 mg/L) to limit microbial growth and account for abiotic losses. Bottles containing the microbial inoculum were autoclaved to quantify the extent of PPCPs sorption to microbes (termed biosorption controls). Sterile nutrients were added to all the bottles to provide excess inorganic nutrients to ensure biodegradation was limited by the ability to use the PPCPs as the carbon and energy source. Filter-sterilized PPCPs were added to the flasks by filter syringes to obtain three different initial concentrations (50, 10, and 1 μg/L). The Erlenmeyer flasks were capped with porous foam caps to facilitate replenishment of oxygen during incubation for 50 days. The batch studies were maintained at room temperature (~25 °C), and were shaken daily to enhance oxygen transfer into the liquid. Active and control microcosms were periodically sampled to quantify the concentrations of the PPCPs remaining. A decrease in PPCP concentration relative to the sterile control concentrations is evidence for biodegradation. The PPCP concentration decrease over time provides an indication of the overall biodegradation rate.

2.8. Biodegradation sample analysis

PPCP concentrations in the biodegradation experiments were determined by withdrawing 6 mL from the batch flasks using sterile syringes. The biological solutions were passed through Milli-pore™ syringe filters (0.22 μM) into 20 mL test tubes. An equal volume of acetonitrile was added, and the derivatization procedure described above was followed except only 150 μL of 10% K_2CO_3 (adjusted to pH 10.5) was added to the water–acetonitrile solvent mixture. Sample concentrations of the PPCPs were determined using the method outlined above.

2.9. BIOWIN predictive program

The relative biodegradabilities of target PPCPs were predicted using the Biodegradation Probability Program for Windows (BIOWIN) developed by the U.S. Environmental Protection Agency (U.S. EPA, http://www.epa.gov/oppt/exposure/docs.e-pisuited1.htm). BIOWIN calculates the probability of rapid or slow biodegradation for a given chemical under aerobic conditions with mixed cultures of microorganisms. BIOWIN divides up the target compound into several fragments based on its structure. The BIOWIN program then uses several approaches to assign a biodegradability to each fragment and sums the fragment values to assign an overall biodegradability to the compound. The approaches to determine the fragment values are based on multiple linear and non-linear regressions in a given database. BIOWIN version 4.1 also includes two new predictive regression models that incorporate the Japanese MITI (Ministry of International Trade and Industry) biodegradation database. The developed linear and nonlinear models (BIODEG) use 186 chemicals as the dataset, while the MITI models use approximately 900 discrete substances as the dataset to determine their regression. Each one of the models in BIOWIN was performed separately to estimate the biodegradability of our target PPCPs, and the BIOWIN results were compared to the batch measurements of relative biodegradability.

Table 2 – Measured concentrations of target PPCPs (ng/L) sorted by therapeutic class in influent and effluent grab samples collected from the BRWWTP, Baltimore, MD

Target PPCP	Therapeutic class	Wastewater influent concentration (ng/L)	Wastewater effluent concentration (ng/L)	Removal efficiency (%)
Valproic acid	Anticonvulsant	140	ND	>99
Gabapentin	Anticonvulsant	100	ND	>99
Phenytoin	Anticonvulsant	450	250	44
Secobarbital	Barbiturates	ND	ND	NA
Phenobarbital	Barbiturates	70	ND	>99
Ibuprofen	NSAID	1900	250	87
Acetaminophen	NSAID	960	ND	>99
Naproxen	NSAID	3200	380	88
Ketoprofen	NSAID	1200	280	77
Diclofenac	NSAID	110	90	18
Gemfibrozil	Antilipemic	410	130	68
5-Fluorouracil	Antineoplastic	ND	ND	NA
4-Chloro-*m*-cresol	Antiseptics	600	ND	>99
Biosol	Antiseptics	250	ND	>99
PCMX	Antiseptics	400	80	80
Biphenylol	Antiseptics	900	100	80
Chlorophene	Antiseptics	750	200	73
Triclosan	Antiseptics	800	250	69

Relative standard deviations (R.S.D.) values were calculated using quadruplicate wastewater influent and effluent samples. Removal efficiency was calculated using the average influent and effluent PPCP concentrations.

3. Results and discussion

3.1. Occurrence in wastewater samples

As shown in Table 2, 16 of the 18 target analytes were detected in the sewage treatment plant influent; only 5-fluorouracil and secobarbital were not detected. Concentrations of PPCPs in the influent ranged from 70 to 3200 ng/L. Non-steroidal anti-inflammatory drugs (NSAIDs) tended to have higher concentrations (e.g., naproxen at 3200 ng/L and ibuprofen at 1900 ng/L) in the influents compared to the concentrations of the other therapeutic classes. These results are not surprising since NSAIDs are known to have high usage (Murray, 2003). Diclofenac, a NSAID, was detected at much lower concentration than the other NSAIDs found in both the influent and the effluent samples. This result is inconsistent with European studies where diclofenac concentrations were found to be one or two orders of magnitude higher than those of the other NSAIDs (Buser et al., 1998; Heberer et al., 1998; Ternes, 1998; Tixier et al., 2003). One explanation for this difference is that diclofenac usage in the U.S. is not as prevalent as its usage in Europe (Murray, 2003).

The occurrence of triclosan in environmental media has been the main focus of studies of commonly used antiseptics (Halden and Paull, 2005; Lindstrom et al., 2002; Poiger et al., 2003; Singer et al., 2002). The phenolic antiseptics tested (e.g., chlorophene and biphenylol) have similar chemical structures to triclosan and were detected at similar concentrations (Table 2). Anticonvulsant drugs, such as valproic acid and phenytoin, were detected at concentrations ranging from 140 to 500 ng/L. These drugs are of concern due to high prescription volume and potential toxicity to humans and ecosystems (Murray, 2003). For nearly all of the PPCPs detected in the BRWWTP influent, effluent concentrations were significantly lower than influent concentrations (Table 2). Ten of the 18 target PPCPs exhibited incomplete removal (removal less than 99%). Only valproic acid, 4-chloro-*m*-cresol, biosol, gabapentin, acetaminophen, and phenobarbital had near complete (>99%) removal. NSAIDs, such as naproxen and ibuprofen, exhibited high (~90%) removal efficiency, yet due to the high influent concentrations of these drugs, appreciable amounts (~200 ng/L) of the drugs were found in the BRWWTP effluent.

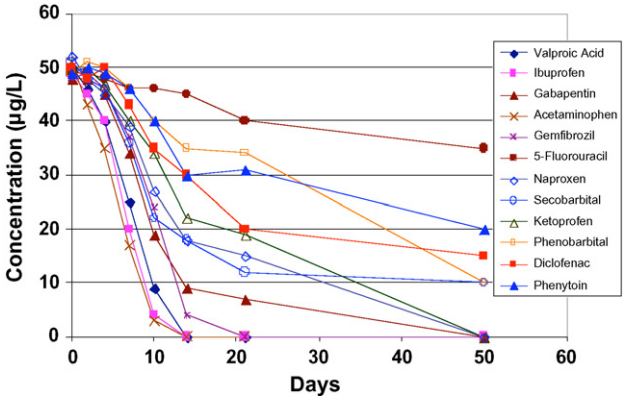


Fig. 1 – Aerobic batch biodegradation of selected pharmaceuticals (initial concentrations of 50 μg/L) inoculated with diluted waste activated sludge.

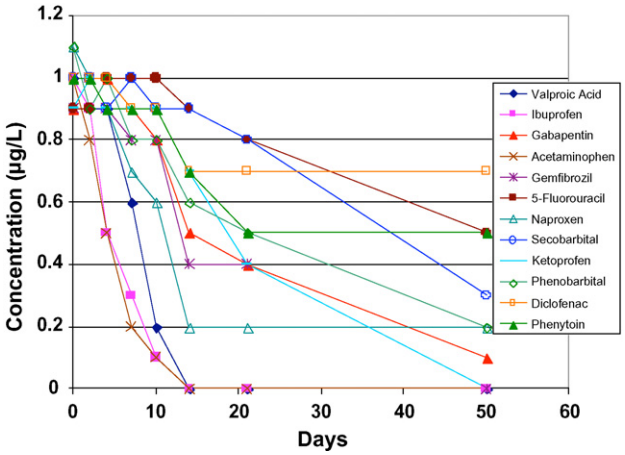


Fig. 2 – Aerobic batch biodegradation of selected pharmaceuticals (initial concentrations of 1 μg/L) inoculated with diluted waste activated sludge.

Phenytoin and diclofenac exhibited less than 50% removal, suggesting their persistence throughout the wastewater treatment processes. In general, there was no clear trend for relating the observed PPCP removals to drug classification or structure.

3.2. Biotransformation of pharmaceuticals

In the abiotic control experiments, the concentrations of PPCPs remained relatively constant over the entire experimental period of 50 days (data not shown). Consequently, abiotic losses were insignificant for the suite of PPCPs tested. Furthermore, biosorption studies revealed little PPCP sorption in the presence of the microbial inoculum (data not shown), indicating that biotransformation was responsible for the observed decreases in PPCP concentrations over the course of the incubation period. For aerobic biodegradation experiments of selected pharmaceuticals, 8 of the 12 drugs tested exhibited biotransformations greater than 80% after 50 days of incubation (Figs. 1 and 2). Valproic acid, ibuprofen, and acetaminophen showed fast biotransformation rates and nearly complete biotransformation following 14 days of incubation. Phenytoin, 5-fluorouracil, and diclofenac were the only three pharmaceuticals with removals of less than 60% after 50 days of incubation. The majority of the drugs exhibited a lag phase of 4 days before any significant removal (>30%) was observed, except for ibuprofen and acetaminophen in which 50% of the parent compound was biotransformed by day 4 (Fig. 2). The same lag phase period was observed for different initial concentrations. As shown in Fig. 2, biotransformation trends for the pharmaceuticals with an initial concentration of 1 μg/L (Fig. 2) were similar to the results with an initial concentration of 50 μg/L (Fig. 1).

3.3. Biotransformation of antiseptics

Batch control experiments with the antiseptics exhibited similar behavior to the studies with pharmaceuticals. The concentrations of the antiseptics remained near the initial

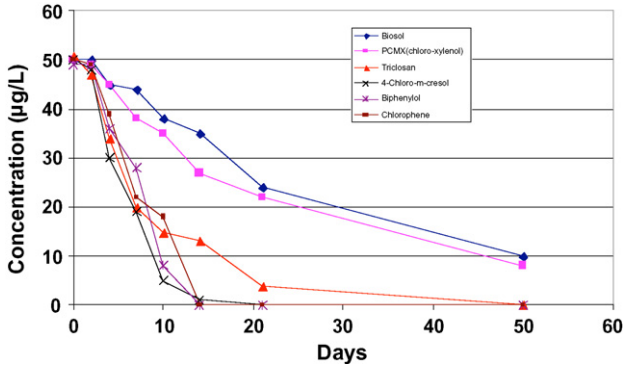


Fig. 3 – Aerobic batch biodegradation of antiseptics (initial concentrations of 50 μg/L) inoculated with diluted waste activated sludge.

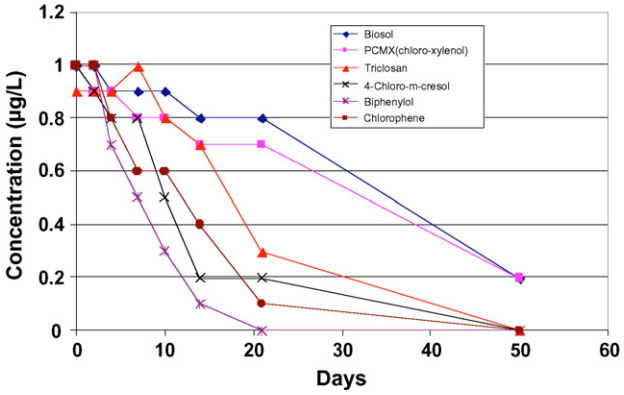


Fig. 4 – Aerobic batch biodegradation of antiseptics (initial concentrations of 1 μg/L) inoculated with diluted waste activated sludge.

concentrations throughout the 50 days of incubation time (data not shown), indicating that no other physical or chemical transformations had occurred. Biosorption experiments also indicated that little sorption occurred to the microbial biomass. At an initial concentration of 50 μg/L and aerobic conditions, four out of six antiseptics exhibited biotransformations greater than 30% in 4 days (Fig. 3). 4-Chloro-*m*-cresol, biphenylol, and chlorophene exhibited greater than 80% biotransformation after 14 days of incubation, indicating relatively fast biotransformation rates for these three compounds. Slower biotransformation rates were observed for biosol and PCMX as they exhibited less than 60% biotransformation after 21 days of incubation. With additional incubation time of 50 days, all of the antiseptics tested were biotransformed greater than 80%. Biotransformation trends for the antiseptics with an initial concentration of 1 μg/L (Fig. 4) were similar to the results with an initial concentration of 50 μg/L (Fig. 3).

3.4. *Predicting biodegradability*

Batch biodegradation experiments are used as a screening tool for biodegradability, but these experiments are often time consuming, laborious, and costly. Quantitative structure–property relationships (QSRP) have been proposed to predict biodegradability as an alternative to conducting batch measurements (Boethling et al., 2003). The BIOWIN software developed by the U.S. EPA has shown positive results for predicting the biodegradation behavior for some antibiotics and pharmaceuticals (Boethling et al., 2004). The possible

Table 3 – Predicted biodegradability of the selected PPCPs (sorted by therapeutic class) using the BIOWIN software

Target PPCP	Therapeutic class	BIODEG linear model	BIODEG non-linear model	MITI linear model	MITI non-linear model	Batch study results % biotransformed
Valproic acid	Anticonvulsant	✓	✓	✓	✓	>99 (✓)
Gabapentin	Anticonvulsant	✓	✓	✓	✓	90 (✓)
Phenytoin	Anticonvulsant	✓	✓	×	×	50 (×)
Secobarbital	Barbiturates	×	×	×	×	70 (×)
Phenobarbital	Barbiturates	✓	✓	×	×	80 (✓)
Ibuprofen	NSAID	✓	✓	×	×	>99 (✓)
Acetaminophen	NSAID	✓	✓	×	✓	>99 (✓)
Naproxen	NSAID	✓	✓	×	×	80 (✓)
Ketoprofen	NSAID	✓	✓	×	×	>99 (✓)
Diclofenac	NSAID	✓	✓	×	×	30 (×)
Gemfibrozil	Antilipemic	✓	✓	✓	✓	>99 (✓)
5-Fluorouracil	Antineoplastic	✓	✓	×	×	50 (×)
4-Chloro-*m*-cresol	Antiseptics	✓	✓	×	×	>99 (✓)
Biosol	Antiseptics	✓	✓	×	×	80 (✓)
PCMX	Antiseptics	✓	✓	×	×	80 (✓)
Biphenylol	Antiseptics	✓	✓	×	×	>99 (✓)
Chlorophene	Antiseptics	✓	✓	×	×	>99 (✓)
Triclosan	Antiseptics	×	×	×	×	>99 (✓)

✓: readily biodegradable; ×: non-readily biodegradable. Readily biodegradable for batch experiments (✓ in the last column) was defined as the compounds which exhibited greater than 80% biotransformation, while non-readily biodegradable means (× in the last column) the compounds which exhibited less than 80% biotransformation.

utility of BIOWIN for estimating aerobic biodegradation of our selected PPCPs was evaluated in this study.

The BIOWIN model simulations are shown in Table 3 along with the measured biodegradation results. Both the MITI linear and non-linear models suggested that most of the selected pharmaceutical and antiseptics were not biodegradable. The MITI linear model only predicts valproic acid, gabapentin and gemfibrozil to be biodegradable while the rest of the target analytes were predicted to be non-biodegradable. In contrast, the BIODEG linear and non-linear models suggested that nearly all of the selected pharmaceuticals (except secobarbital) and antiseptics (except triclosan) were biodegradable. When comparing our experimental data (using 80% biotransformation as a guide for determining readily biodegradability) with all the predicted models, our data seemed to fall in between these models. The non-linear BIODEG program seemed to correspond better with our experimental data in comparison to the other three models. Inconsistencies still existed between the predicted and experimental biodegradation results.

4. Concluding remarks

Using the multi compound analytical method developed for trace levels of PPCPs in wastewaters, 16 of the 18 target PPCPs were detected in the influent to the BRWWTP at concentrations ranging from 70 to 3200 ng/L. BRWWTP effluent concentrations were significantly lower than influent concentrations for nearly all of the PPCPs detected. The extents of removals were highly variable and could not be correlated to drug classification or structure. Aerobic biodegradation studies revealed that many of the target drugs were effectively biodegraded in the aerobic cultures (>80% concentration decrease relative to abiotic controls after 50 days of incubation). All six of the antiseptic compounds exhibited greater than 80% removal after 50 days of incubations. The data sets for initial concentrations of 50, 10, and 1 μg/L were virtually identical, indicating that the biodegradation trends were not sensitive to the broad concentration range tested. The submodels within BIOWIN were not able to match consistently the experimental determinations of PPCP biodegradation; two submodels tended to overestimate biodegradability while the remaining two submodels tended to underestimate biodegradability. Although the occurrence study revealed the presence of PPCPs in wastewater effluents, the biodegradation study suggests that biotransformation is a possible removal mechanism for PPCPs during groundwater recharge or soil aquifer treatment for water recycling.

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